Forces in Biosystems

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- Theory and Biosystems
- Synthetic Biology, Bottom-Up
- Forces Generated by Membranes
- Outlook on Mol Machines
- Perspectives and Challenges

Basic Aspects of Theory

Criteria for a 'good' theory:

- Consistent with fundamental laws
- Consistent with experimental/simulation data
- Helpful to understand experimental/simulation data
- General relations between experimental observables
- Predictive power: interesting, nontrivial predictions

Pleasures of theory:

- Quantitative predictions confirmed by experiment
- New insight into underlying mechanisms
- Aesthetic appeal

Some Quotes

„Nothing is more practical than a good theory“

Immanuel Kant

„As simple as possible but not simpler“

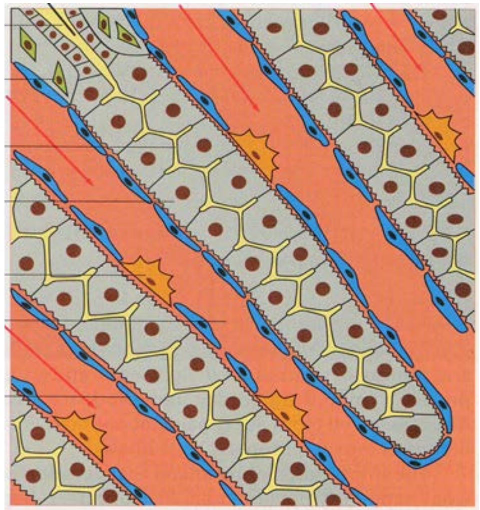
Albert Einstein

„A good theory is like a good joke: it is short and the last line is quite unexpected“

H. L. Friedman

Biosystems: Top-Down

Human body
[m]

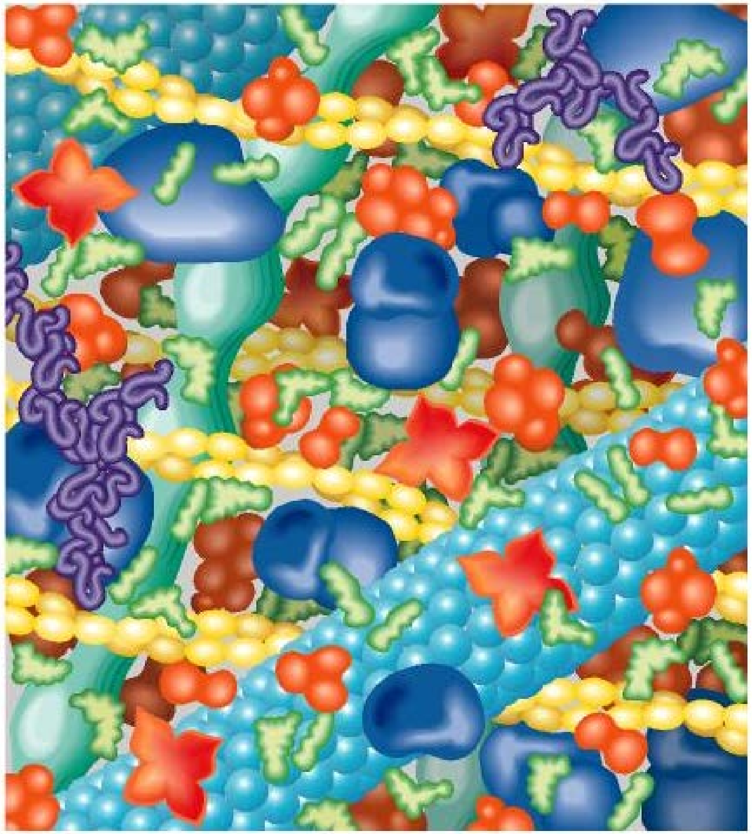


Tissues
[mm]

Cells
[μm]

Molecular
Assemblies
[nm]

Universal Nanostructures



25 nm

Bsp: Filament

- Water and ions
- Small molecules (monomers) form macromolecules such as
  - Proteins
  - RNA, DNA
  - Lipids
  - Polysaccharides
- Macromolecules form molecular assemblies such as
  - Ribosomes
  - Filaments
  - Membranes ....

‘Selforganization’

- Assembly of building blocks "by themselves"
- Instructions from local environment

(1) Selforganization via molecular interactions

Structure formation close to equilibrium

Examples: Protein folding, Binding, Adhesion, ...

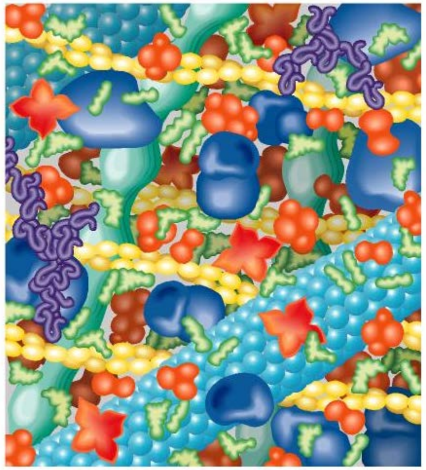
(2) Selforganization via free energy transduction

Chemomechanical coupling far from equilibrium

Examples: Molecular motors, Filament assembly, ...

- Biological systems are difficult to understand because they exhibit ‘entanglement’ of (1) and (2)
- ‘Disentangle’ via biomimetic model systems

Hidden Dimensions



- Molecular and nanoscopic building blocks are

  small + flexible + mobile

  Length scales: nm up to µm

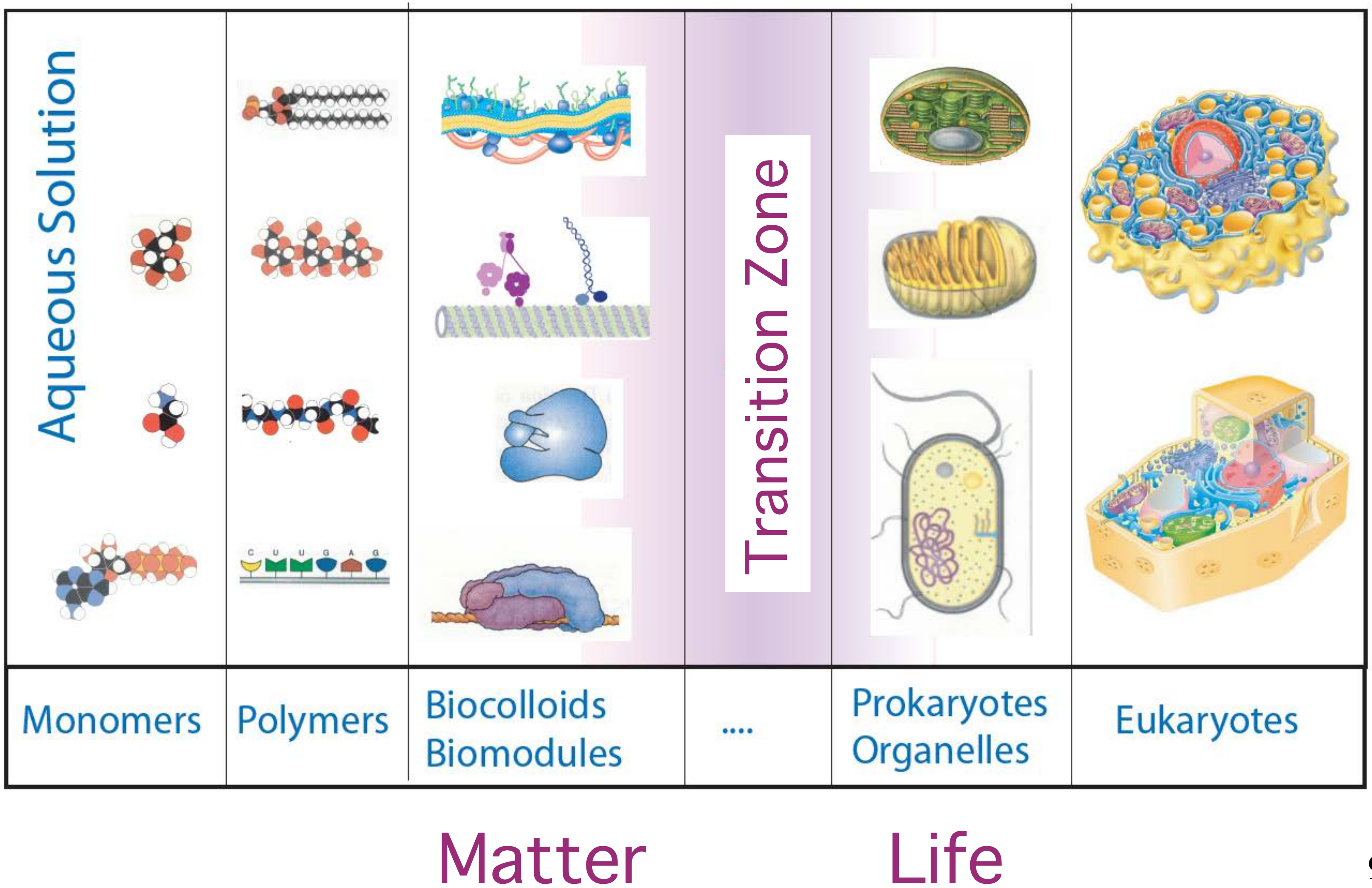
  Time scales: ns up to min

- No single experimental method can cover all scales
- High spatial resolution but low temporal resolution,

  No experimental nanoscope

- High spatio-temporal resolution by simulations
- Theory: Unification of experimental and simulation data

Biosystems: Bottom-Up



Two Routes into Transition Zone

Bottom-Up: Synthetic Cells

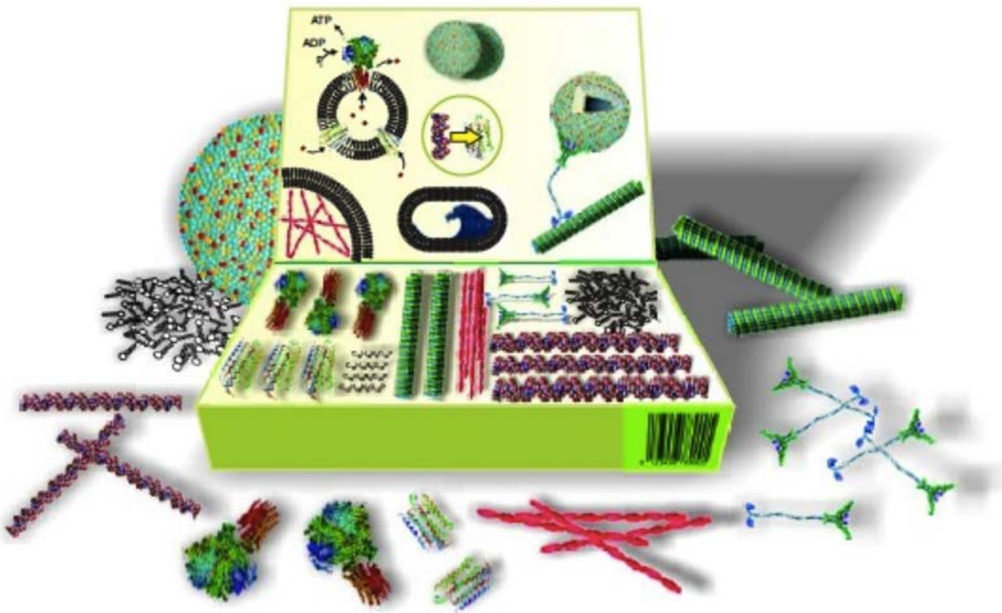
- Develop important building blocks or modules
- Assemble these modules into larger structures
- Integrate more and more modules ...

Top-Down: Minimal Cells

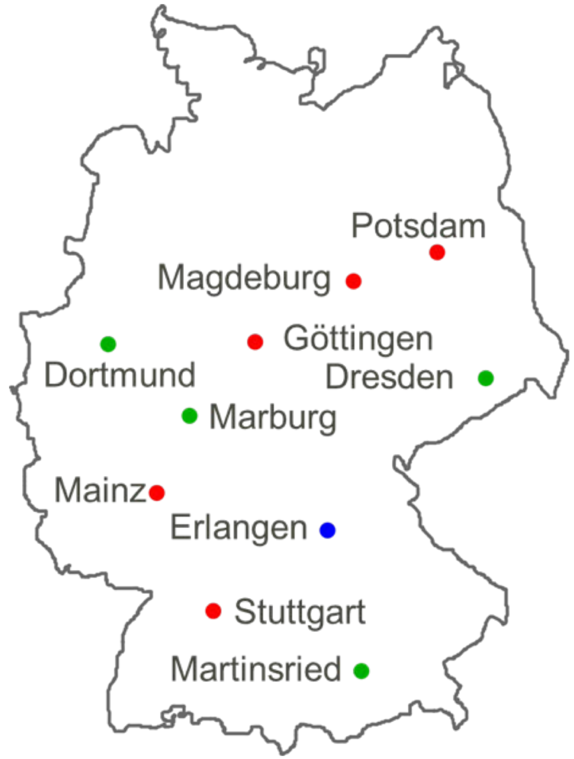
- Start with relatively simple cells
- Eliminate more and more components
- Problem: many remaining genes with unknown functions

Synthetic Biology, Bottom-UP

MaxSynBio Consortium:
Create a toolbox of modules
to build a synthetic cell



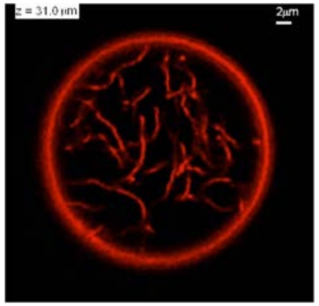
9 Max Planck Institutes:

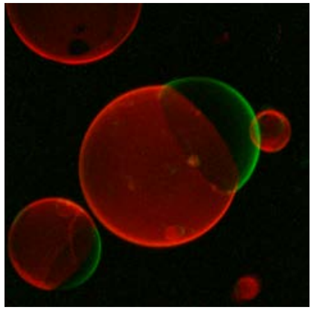


Motivation:
No understanding of minimal
cell from top-down approach

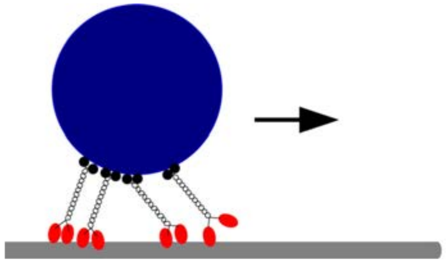
Speakers:
Petra Schwille,
Kai Sundmacher

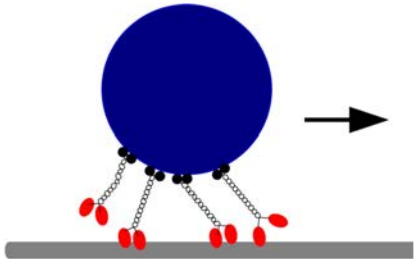
Basic Modules for Synthetic Cells



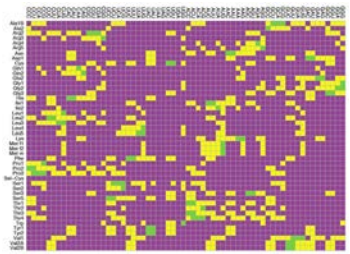


- Membrane and vesicles, fluid compartments, remodeling





- Directed transport by molecular motors, free energy transduction

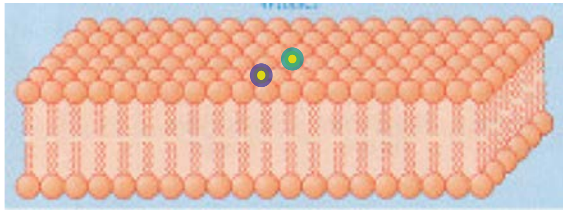


- Template-directed assembly, ribosomes, protein synthesis

Biomembranes are Fluid Bilayers

- Fluid membranes, i.e., fast lateral diffusion:

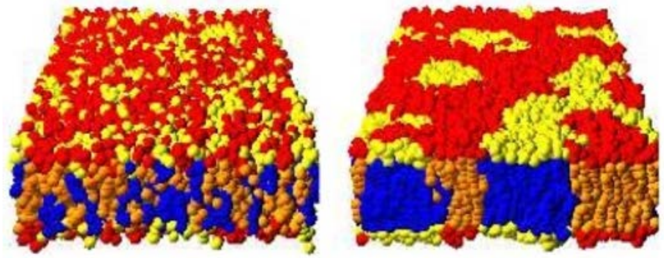
  Diffusion constant ~ μm^2 /s



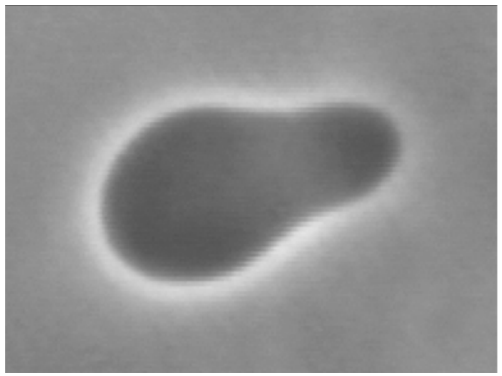
4 nm

lipid swapping ~ ns

- Lateral diffusion => Compositional responses, demixing, domain formation ...



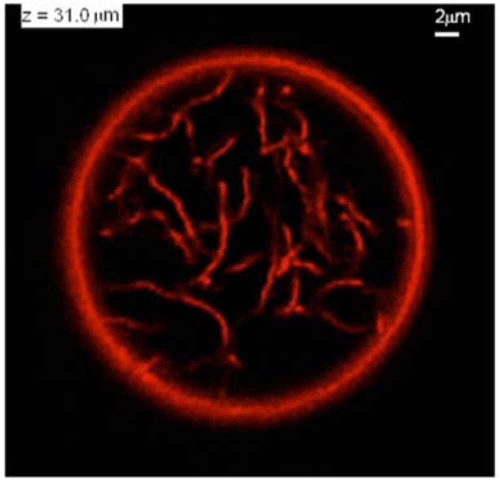
- Flexibility => Morphological responses, budding, tubulation, ... Direct evidence for fluidity



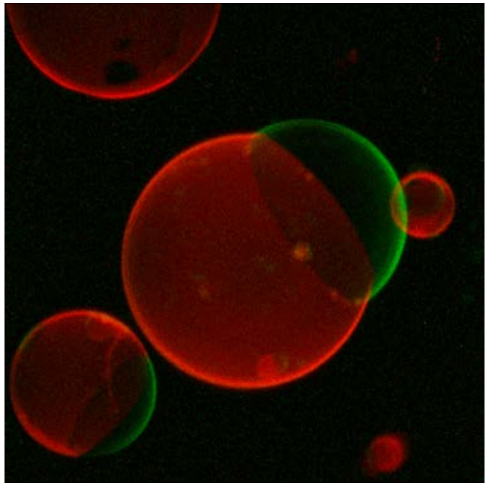
40 μm

Multiresponsive Behavior

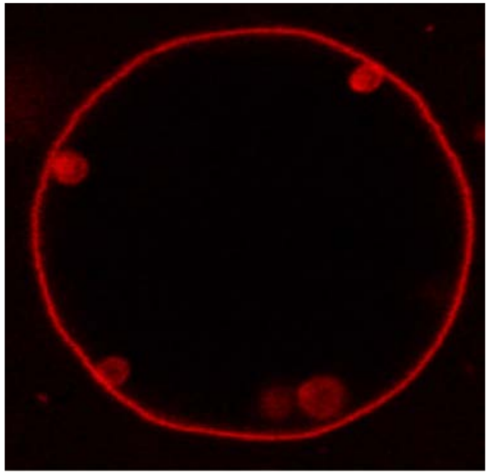
- Giant unilamellar vesicles (GUVs), tens of micrometers
- Remodelling in response to various perturbations:



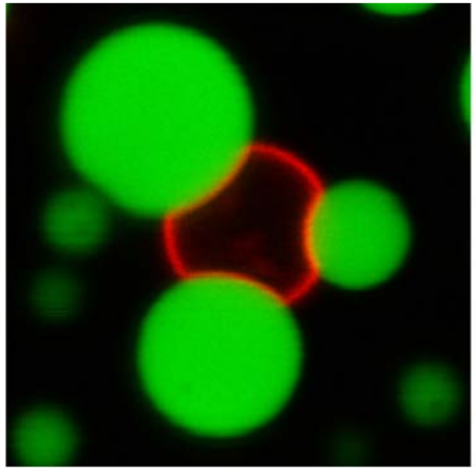
Nanotubes from polymer adsorption, tube width ~ 100 nm



Formation of intra-membrane domains, 2D phase separation



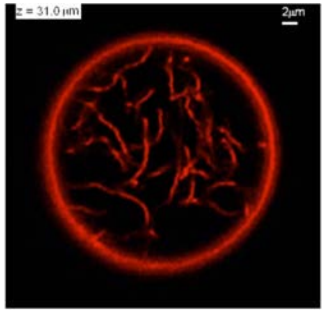
Small buds from adsorption of two ESCRT proteins



Shaping GUVs by membrane-less organelles, FUSb

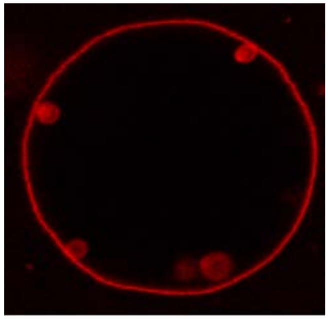
- What are the forces that drive remodelling processes?

Forces Generated by Membranes



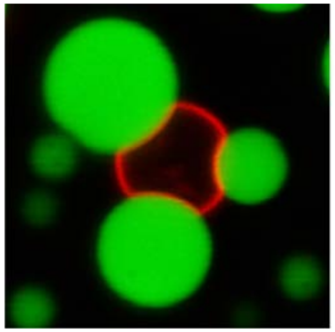
- Spontaneous Tubulation:

  Spont curvature generates spont tension and constriction forces



- ESCRT-induced budding and fission:

  Adhesion-induced constriction forces



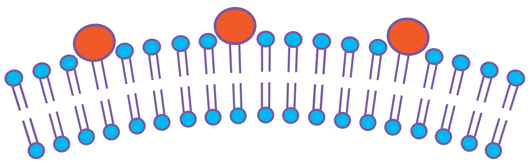
- Interactions with membrane-less organelles:

  Capillary forces and curvature generation

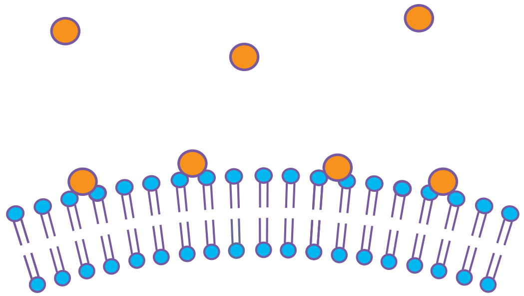
Spontaneous = Preferred Curvature

- Spontaneous or preferred curvature *m* describes bilayer asymmetry = asymmetry between two leaflets

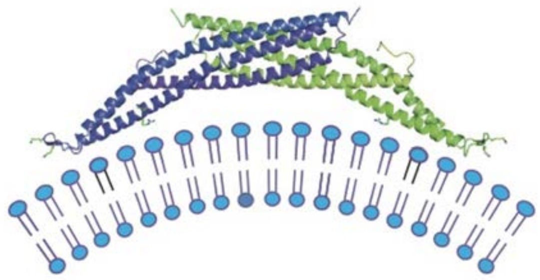
- Different molecular mechanisms for bilayer asymmetry:



Asymmetric composition, e.g., ganglioside



Asymmetric adsorption of small molecules

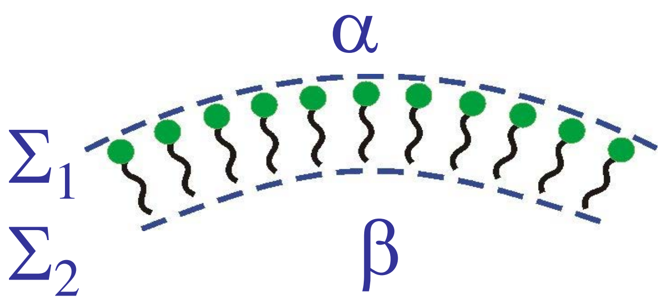


Asymmetric protein coats, e.g. BAR-domain

Concept of Spontaneous Curvature

- W. D. Bancroft (1913)

  ‘Theory of emulsification’



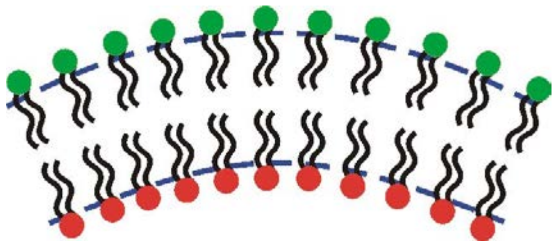
- F. C. Frank (1958)

  ‘On the theory of liquid crystals’

splay term from symmetry arguments

- W. Helfrich (1973)

  ‘Elastic properties of lipid bilayers’

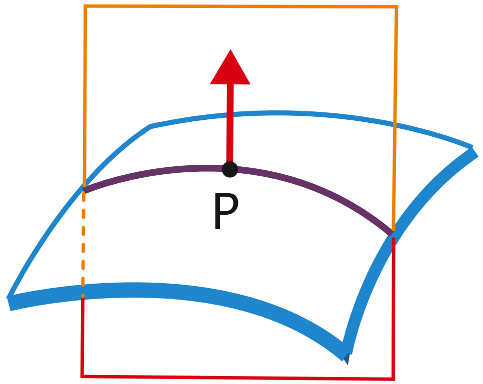


- Variants of curvature models:

  E. Evans, S. Svetina + B. Zeks, M. Wortis

Curvature Elasticity

- Mean curvature *M* tries to adapt to spontaneous (or preferred) curvature *m*



- Curvature or bending energy:

$$E_{cu} = \int dA \, 2 \, \kappa \, (M - m)^2$$

integral over membrane area *A*

- 2nd fluid-elastic parameter: Bending rigidity κ

Dimensions of energy, $\kappa = 10^{-19}$ J = 20 k_B T

- Range of spontaneous curvatures *m*

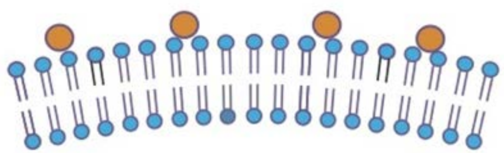
from 1/(20 nm) to 1/(20 µm)

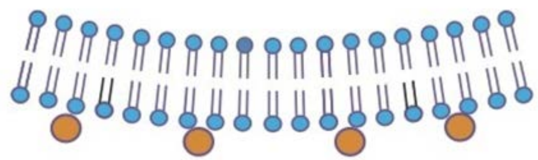
Sign of (Spontaneous) Curvature

- Mean curvature M and spontaneous curvature m
  can be positive or negative
- Sign defined with respect to interior/exterior compartments
  = with respect to inner/outer leaflet

exterior compartment outer leaflet

$M > 0$





$M < 0$

interior compartment inner leaflet

Mean curvature M is positive (negative) if membrane bulges towards exterior (interior) compartment

Shape Functional for Vesicles

- Vesicle has constant surface area A and fixed volume V
- Shape functional:

$$F = E_{\mathrm{cu}} + \Sigma A - \Delta P\, V$$

Mechanical tension (Σ)

Pressure difference (ΔP)

- Consider Σ and ΔP as Lagrange multipliers
- Minimization with respect to normal displacements of membrane, Euler Lagrange equation:

$$\Delta P = 2\,\Sigma M - 2\kappa\, \Delta_{LB} M - 4\kappa\, [M - m]\, [M\,(M + m) - G]$$

- Laplace equation plus κ–dependent terms

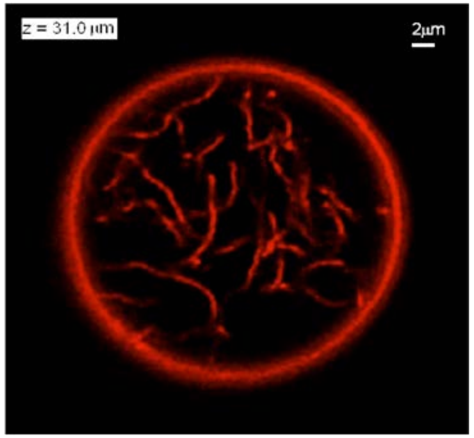
Ou-Yang, Helfrich, *Phys. Rev. A* (1989)

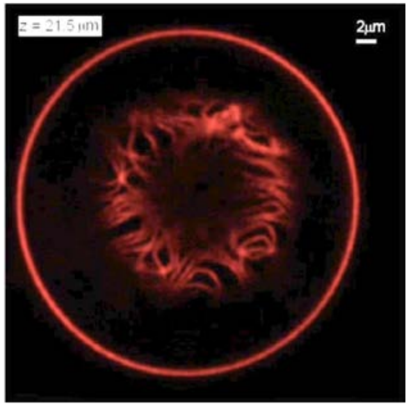
- Explicit solutions in reduced shape spaces

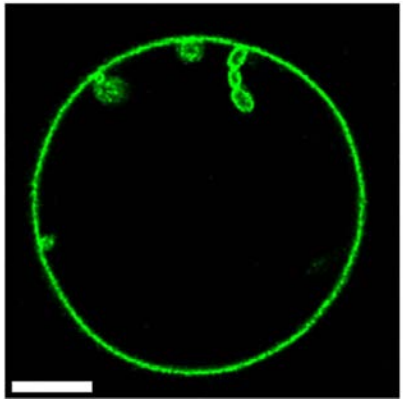
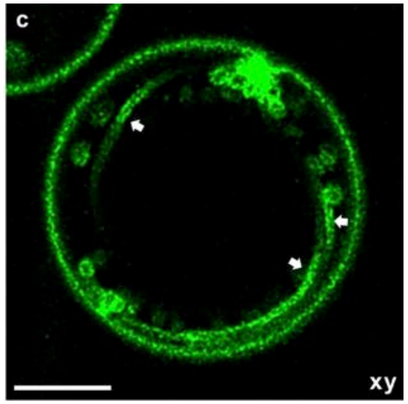
Buds and Nanotubes

Liu et al, *ACS Nano* (2016)

- Lipid mixture of DOPC, DPPC, cholesterol
- Membranes labeled by fluorescent dyes
- Liquid-disordered (red) and liquid-ordered phase (green)





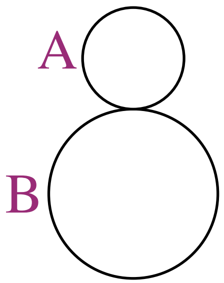



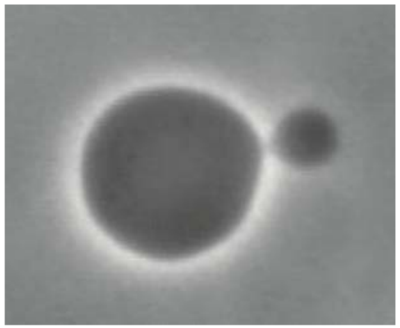
- Asymmetric environment, different PEG concentrations
- Deflation: Bud and tube formation without external forces
- Tubes can be necklace-like or cylindrical

Membrane Necks

- For $m \neq 0$, curvature elasticity leads to spherical membrane segments connected by membrane necks

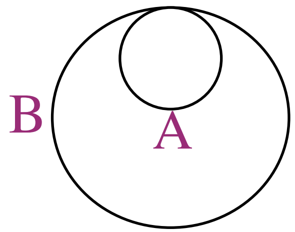
- Out-bud:

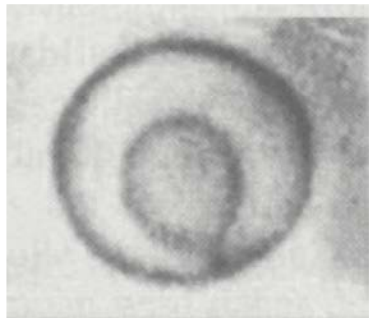




spont curv $m > 0$

- In-bud:





spont curv $m < 0$

- Closed neck is stable if:

$$0 < M^A + M^B \leq 2\,m$$

$$2m \leq M^A + M^B < 0$$

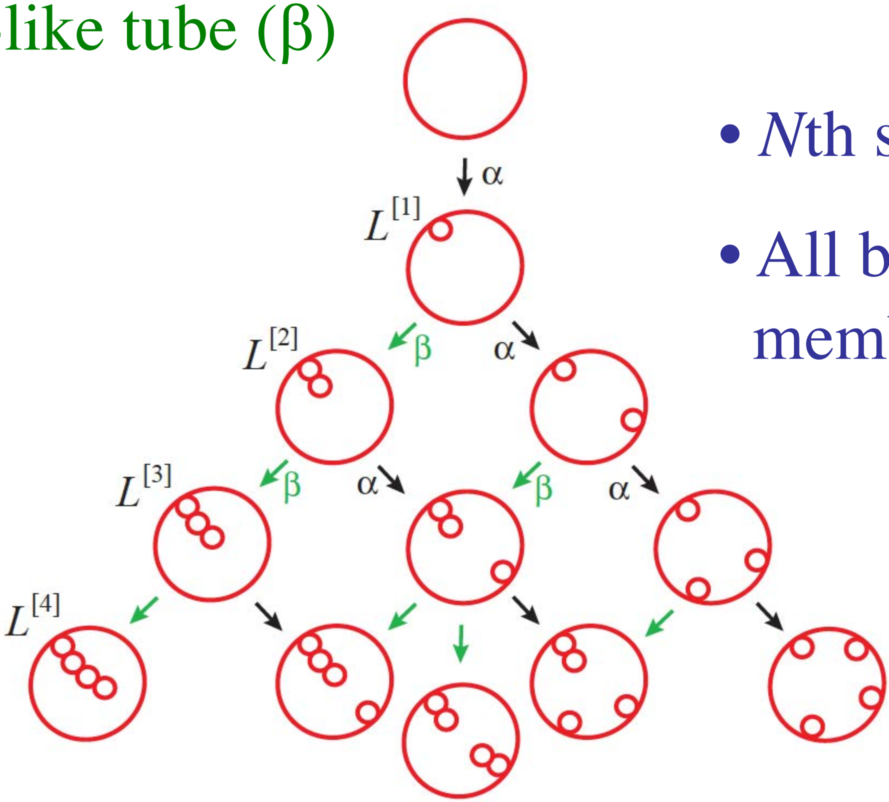
- Relation between geometry and material parameter

Nucleation and Growth of Tubes

- Vesicle membrane with large spont curv *m*

Liu et al, *ACS Nano* (2016)

- Osmotic deflation of GUV in discrete steps
- At each step, nucleation of new bud (α) or extension of necklace-like tube (β)

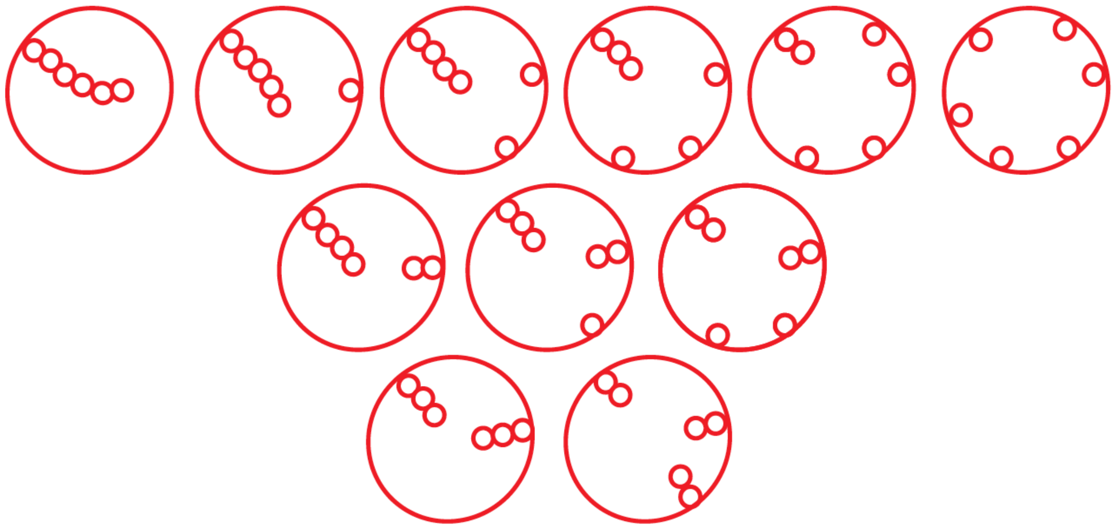


- *N*th step leads to *N* in-beads
- All beads are connected by membrane necks (not visible)

=> Buds are nuclei for necklace-like tubes

Morphological Complexity

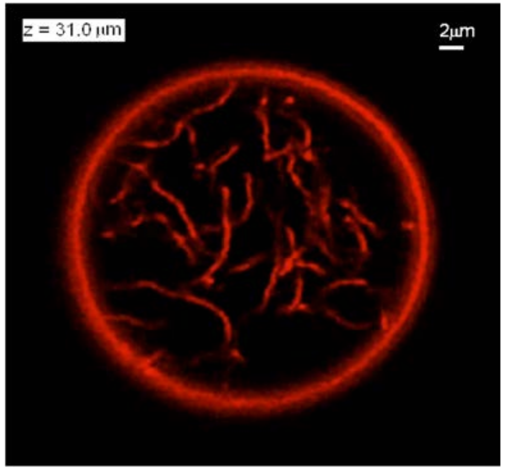
- After 6th step, 11 morphologies with 6 beads:



- All beads are connected by membrane necks
- All morphologies have the same area, volume, and curvature energy
- Rugged energy landscape contains 11 intersecting branches
- For large N, # of N-bead morphologies grows as exp[c $\sqrt{N}$]

Spont Curvature Generates Tension

RL, Faraday Discuss. (2013)



- Tubulation leads to tense mother vesicle
- Total tension in Euler-Lagrange equation has two components:

$$\hat{\Sigma} = \Sigma + \sigma$$

Mechanical tension Σ stretches the membrane

Spontaneous tension $\sigma = 2\,\kappa\, m^2$ curves the membrane

- Presence of nanotubes implies dominance of spontaneous tension, mechanical tension can be ignored
- Example: Spont curvature $\approx -1/(100\ \text{nm})$ implies

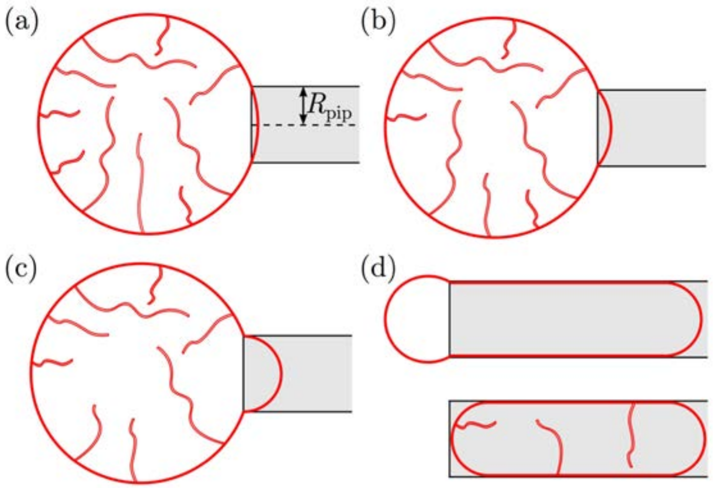
Spontaneous tension $\sigma \approx 10^{-2}$ mN/m

Mechanical tension $\Sigma \approx 10^{-4}$ mN/m

Spont Tension from Experiment

- Retraction of tubes by micropipettes:

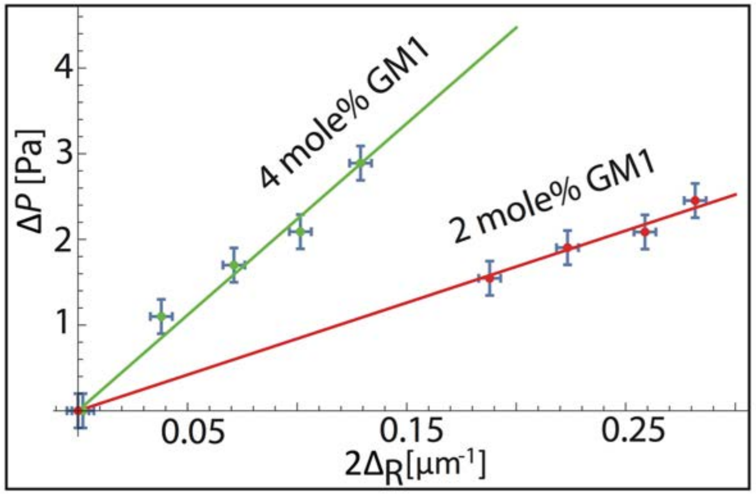
Bhatia et al, ACS Nano (under revision)



Initial aspiration up to hemispherical tongue

then vesicle starts to flow into micropipette, increased robustness !

Aspiration pressure

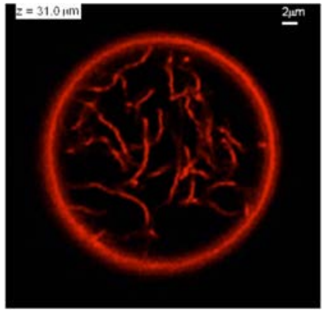


Initial aspiration:

Aspiration pressure versus geometric quantity Δ_R

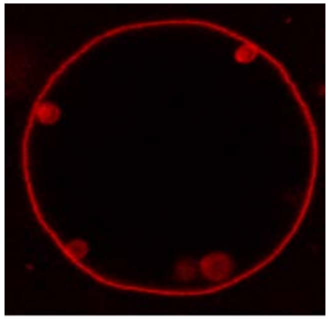
Slope = spontaneous tension σ

Forces Generated by Membranes



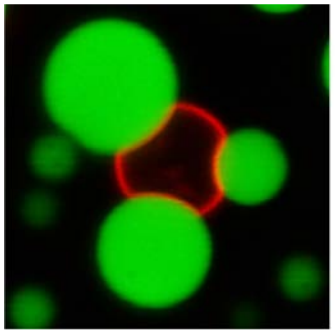
- Spontaneous Tubulation:

  Spont curvature generates spont tension and constriction forces



- ESCRT-induced budding and fission:

  Adhesion-induced constriction forces



- Interactions with membrane-less organelles:

  Capillary forces and curvature generation

Sequential ESCRT Addition

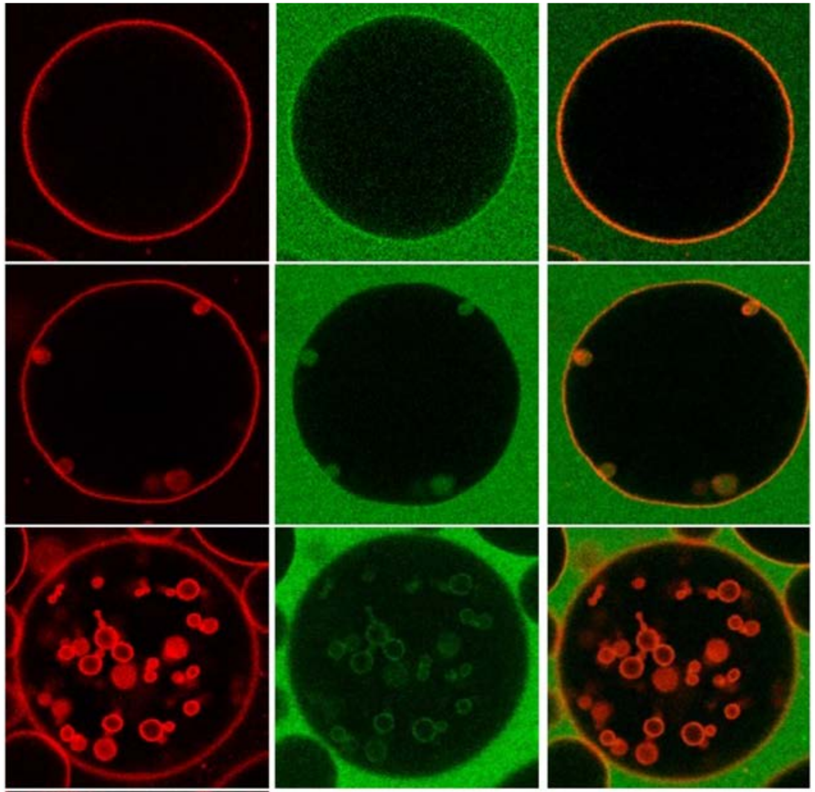
Avalos Padilla et al, *Frontiers Microbiology* (in press)

- Sequential addition of three ESCRT proteins to GUVs:

+ ESCRT 1
Vps20

+ ESCRT 2
Vps32

+ ESCRT 3
Vps24



adsorption of Vps20

multiple buds + necks

fission of necks

Digression on Nanoparticles

Agudo-Canalejo, RL, *ACS Nano* (2015)
Nano Letters (2015)

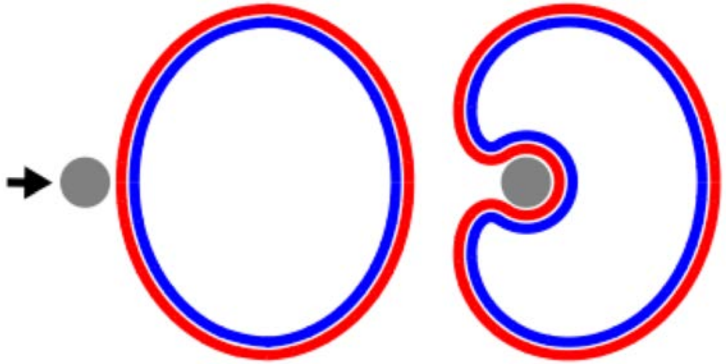

Jaime Agudo

- Nanoparticles interacting with membranes, vesicles and cells:

  biomedical imaging,

  drug delivery, nanotoxicity,

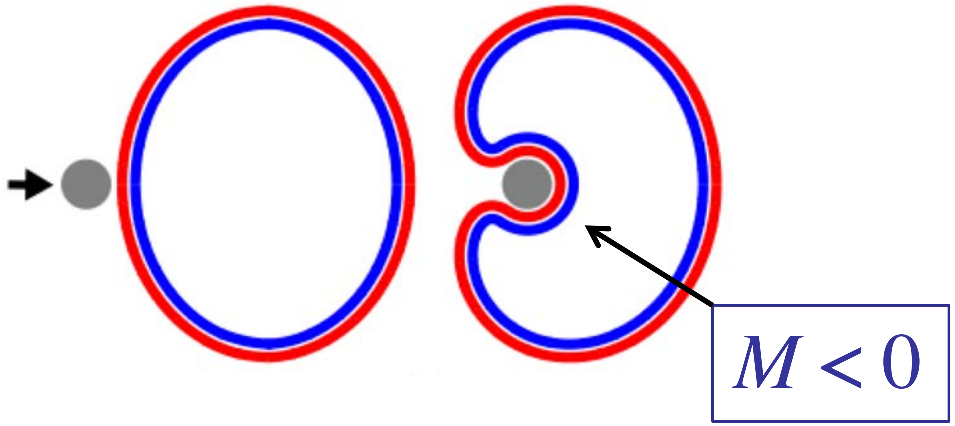
  virus infection ...



- Important control parameters:
  - Adhesive strength W ~ surface chemistry
  - Particle size R_{pa}
  - Spontaneous curvature m

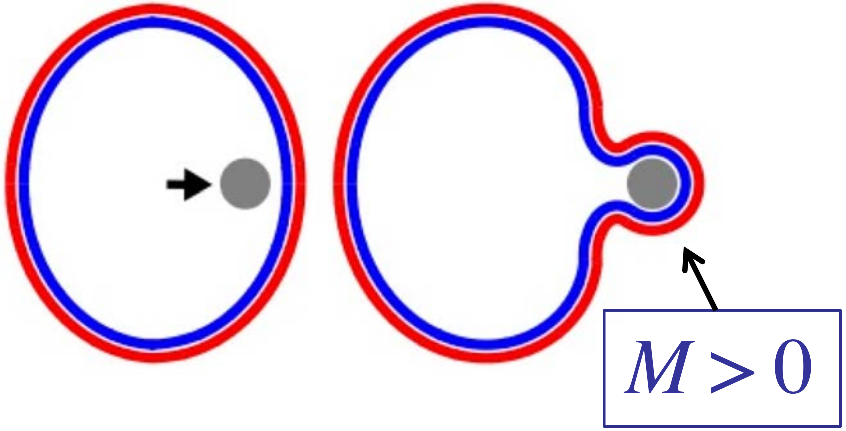
Endo- and Exocytosis

- Endocytic engulfment:



- Particles originate from exterior compartment
- Negative curvature M of bound membrane segment
- Favored by $m < 0$

- Exocytic engulfment:



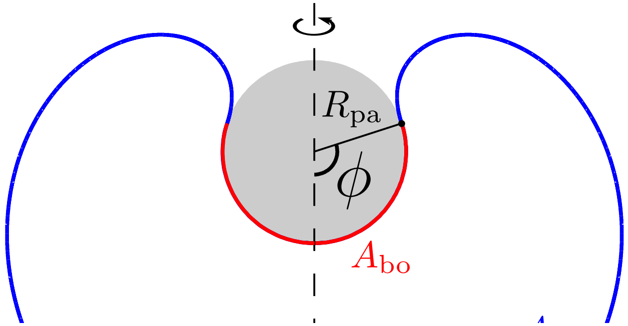
- Particles originate from interior compartment
- Positive curvature M of bound membrane segment
- Favored by $m > 0$

Shape Functional with Adhesion

- Shape functional with adhesive term:

Adhesion (free) energy

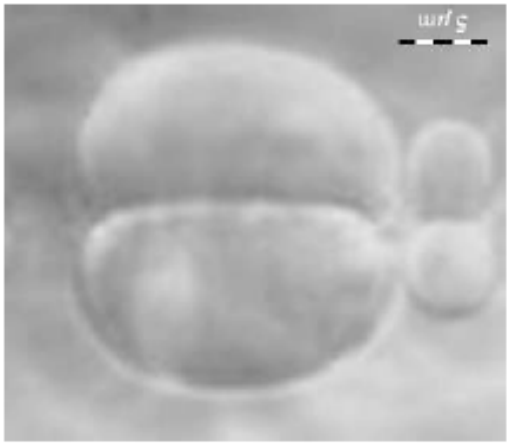
$$F = E_{cu} + \Sigma A - \Delta P\ V - W A_{bo}$$



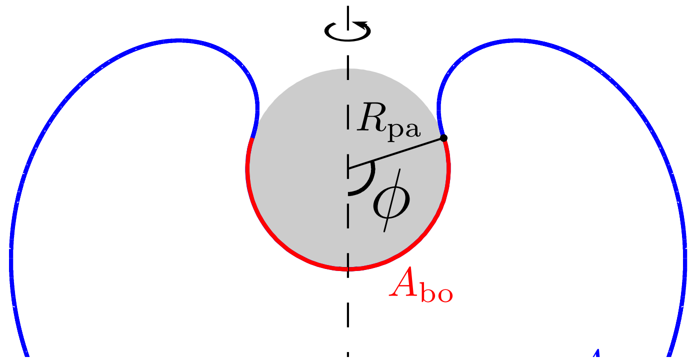
Adhesive strength $W > 0$
Area A_{bo} of bound membrane segment
Boundary bound / unbound segment

- Competition between adhesion and bending encoded in adhesion length $R_W = (2\kappa/W)^{1/2}$
- Contact mean curvature M_{co} for membrane adhesion to planar surface:

$$M_{co} = 1/R_W = (W/2\kappa)^{1/2}$$



Contact Mean Curvature at Particle



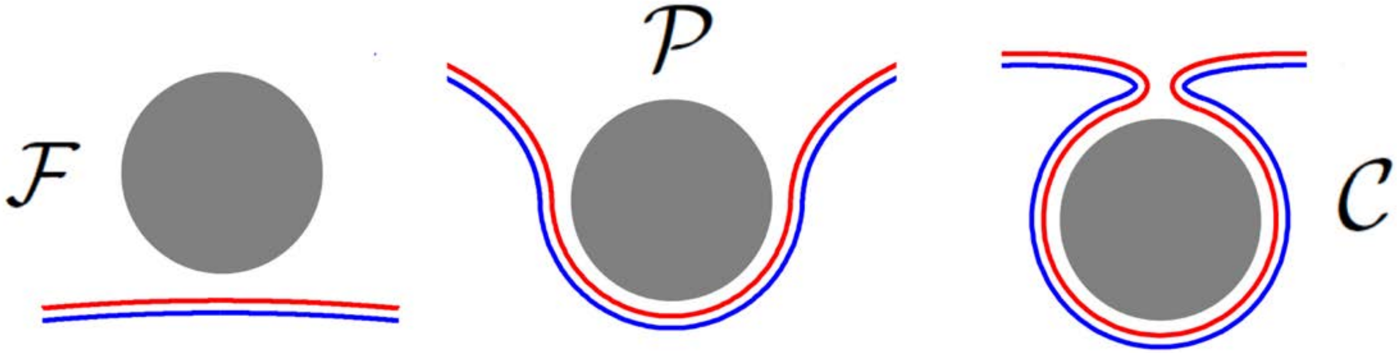
- Membrane adhering to particle
- Bound membrane segments (red) follows particle surface
- Contact line provides boundary condition for unbound segment (blue)
- Principical curvatures along contact line:

$$C_1 = (2W/\kappa)^{1/2} - 1/R_{pa} \quad \text{(along contour)}$$

$$C_2 = -1/R_{pa} \quad \text{(perp to contour)}$$

- Contact mean curvature: $$M_{co} = 1/R_W - 1/R_{pa}$$
- Independent of spontaneous curvature m !

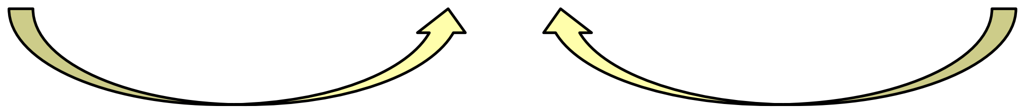
(In)Stability of Particle States



Free

Partially engulfed

Completely engulfed



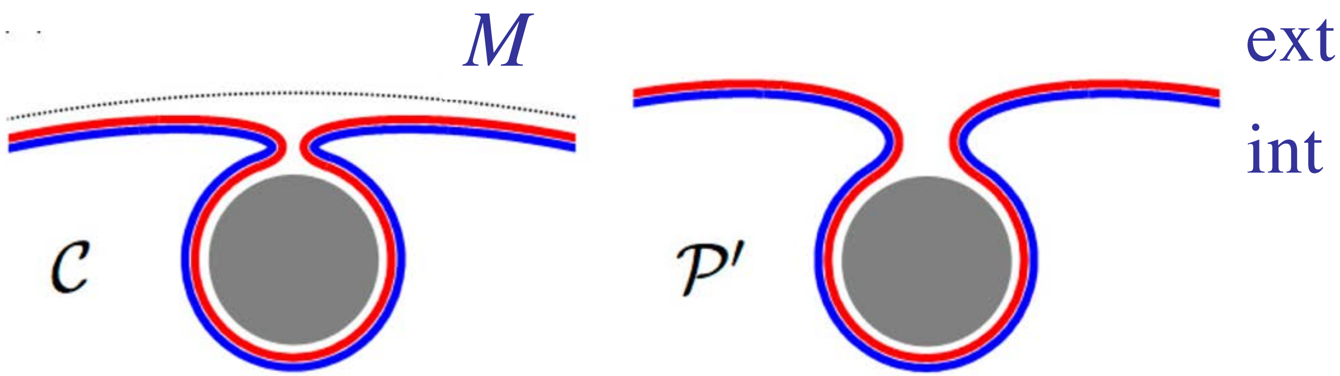
Onset of adhesion

Opening of neck

- Competition between adhesion and bending:

Adhesion length $R_W = (2\kappa/|W|)^{1/2}$

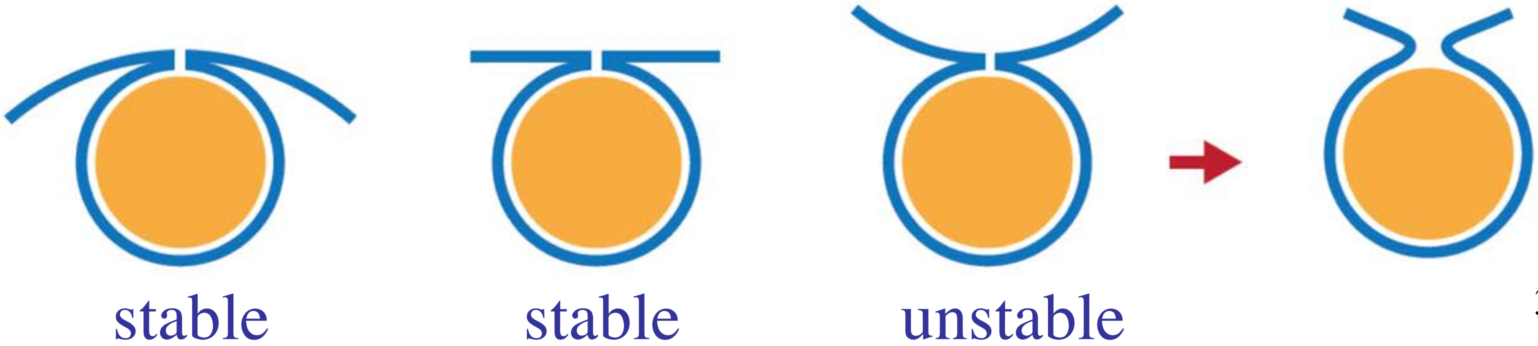
Opening of Membrane Neck



- Closed neck is stable if mean curvature

$$M \geq \quad 2\,m - M_{co} = 2\,\mathrm{m}\ - 1/R_{\mathrm{W}} + 1/R_{\mathrm{pa}}$$

- Example: threshold value $2\,m - M_{co} = 0$:

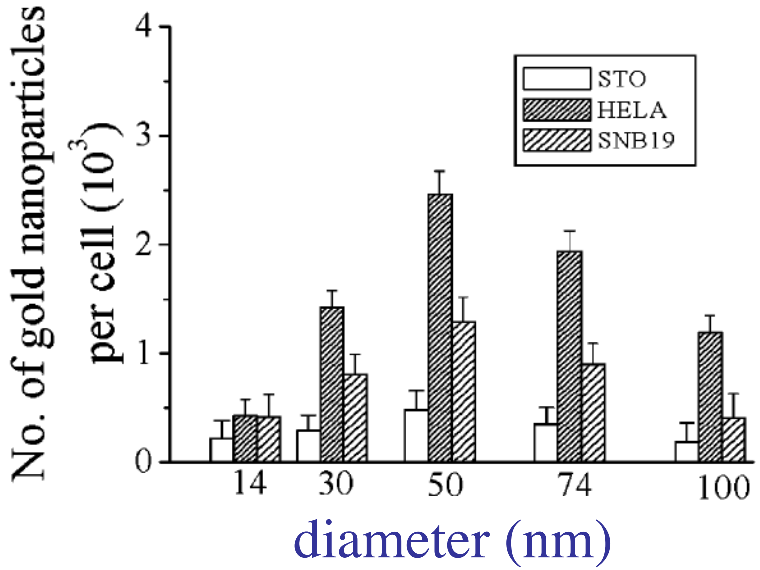


Clathrin-dependent Endocytosis

Chithrani et al, *Nano Letters* (2007)

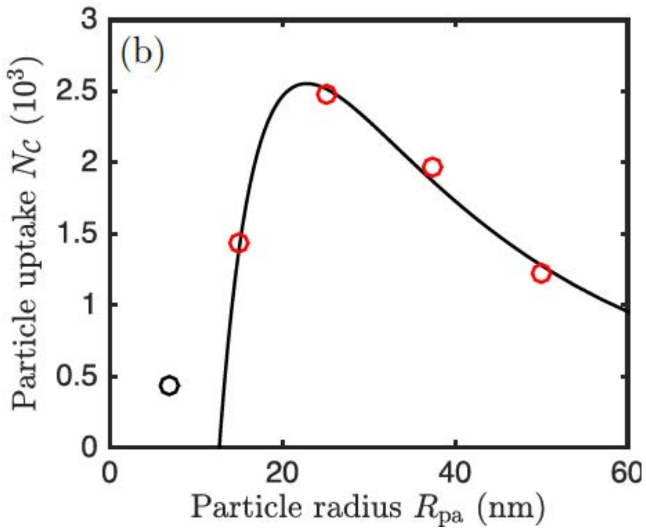
- Uptake of gold nanoparticles by cells
- Particles bind to transferrin receptors
- Assembly of clathrin-coated vesicles

Non-monotonic size-dependence !



- Cell membrane with two types of segments, bound and unbound
- Bound segment contains protein coat with spont curv m_{bo} = - 1/(40 nm)
- Good agreement with exp data:

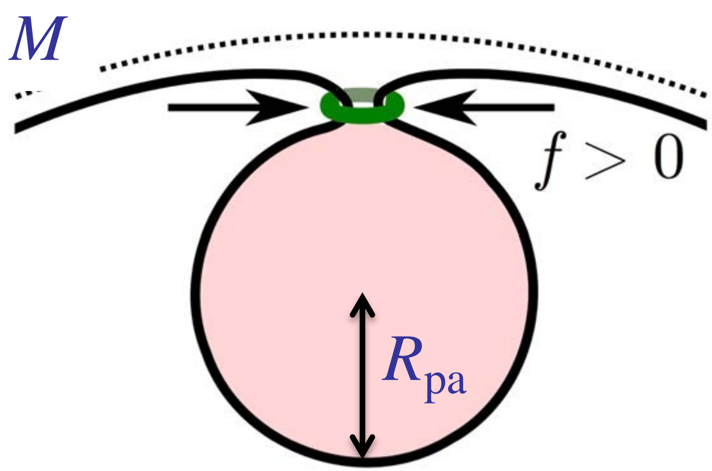
Agudo-Canalejo, RL: *ACS Nano* (2015)



Stability of Membrane Necks

Agudo-Canalejo, RL, *Soft Matter* (2016)

- Necks of in-buds stabilized by
  - constriction force f
  - (negative) spontaneous curvature m
  - substrate with adhesive strength W



- General stability relation for m < 0:

$$M_{pa} = -1/R_{pa}$$

$$f\,(4\pi\kappa)^{-1} \;-\; 2\,m \;+\; (|W|/2\kappa)^{1/2} \;-\; 1/R_{pa} + M \;\geq\; 0$$

- Linear superposition of different mechanisms

Effective Constriction Force

- General stability relation for m < 0:

$$f\,(4\pi\kappa)^{-1} - 2\,m + (|W|/2\kappa)^{1/2} - 1/R_{pa} + M \geq 0$$

- Effective constriction force

$$f_{eff} = f + 8\,\pi\,\kappa\,|m| + 2\,\pi\,(2\,\kappa\,|W|)^{1/2}$$

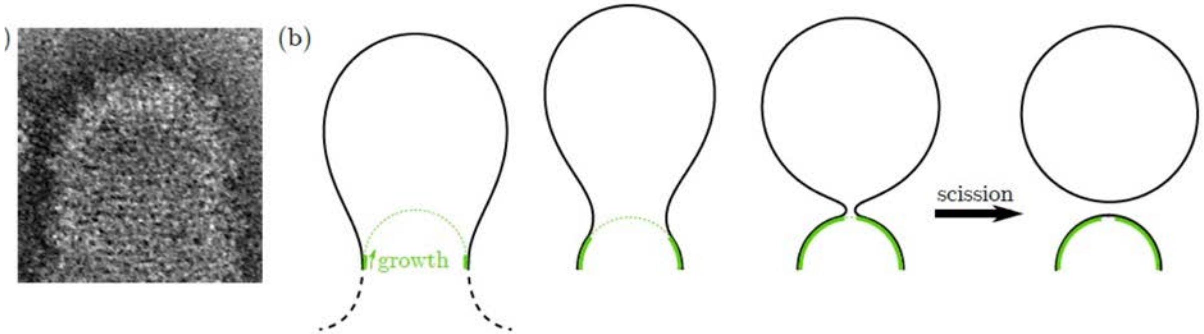
- Spontaneous curvature generates force $f_m = 8\,\pi\,\kappa\,|m|$
- Example: Spont curvature $\approx -1/(100\text{ nm})$ implies
  curvature-induced constriction force $f_m \approx 25$ pN
- Adhesion-induced constriction force $f_W = 2\,\pi\,(2\,\kappa\,|W|)^{1/2}$

ESRCT-induced Fission

- ESCRTs assemble at inner leaflet of cell membrane:

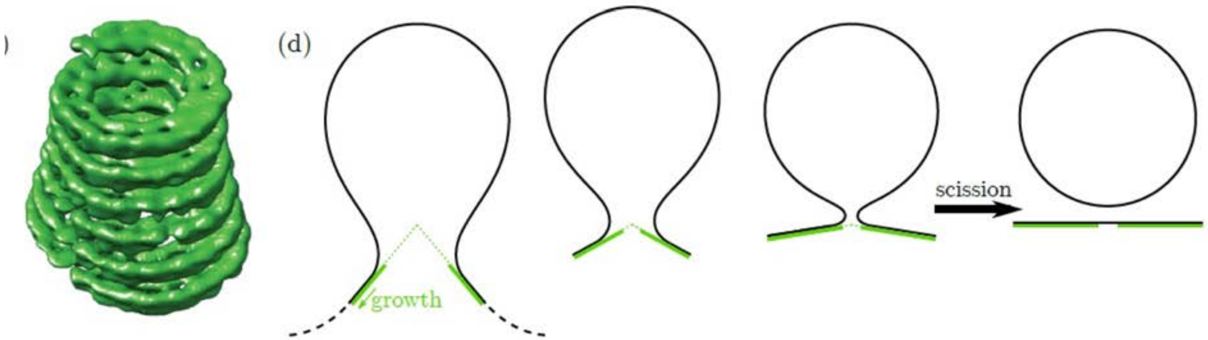
- Domes:

Fabrikant et al,
PLoS CB (2009)



- Cones:

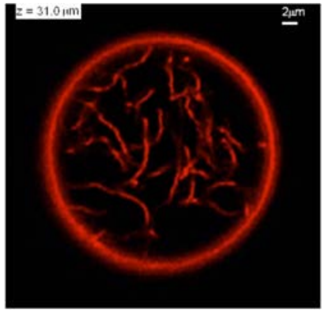
Schöneberg et al,
Nature RMCB (2017)



Agudo-Canalejo, RL, (under review)

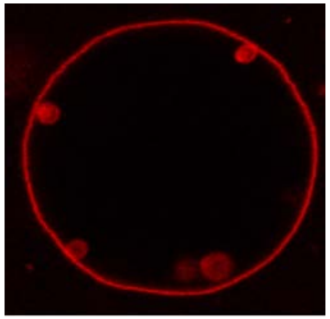
- Closure via cones is energetically more favorable
- Cones generate constriction force $f_W \approx 100$ pN

Forces Generated by Membranes



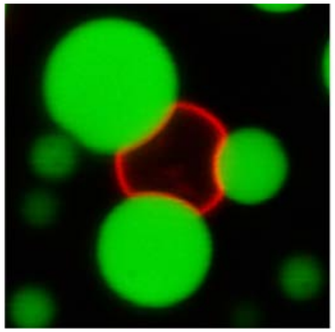
- Spontaneous Tubulation:

  Spont curvature generates spont tension and constriction forces



- ESCRT-induced budding and fission:

  Adhesion-induced constriction forces



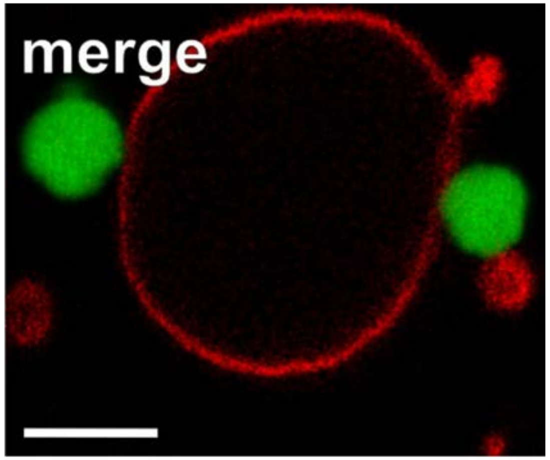
- Interactions with membrane-less organelles:

  Capillary forces and curvature generation

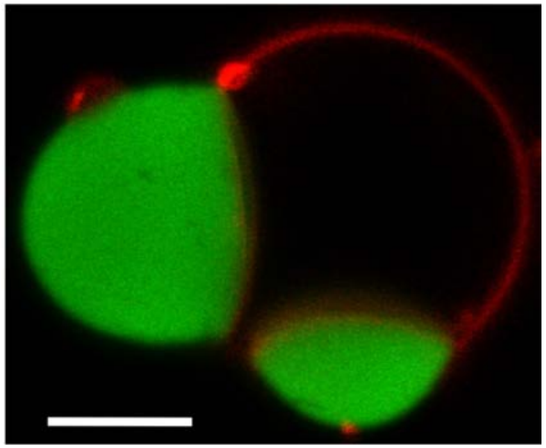
Membrane-less Organelles

Brangwynne ... Hyman, *Science* (2009)

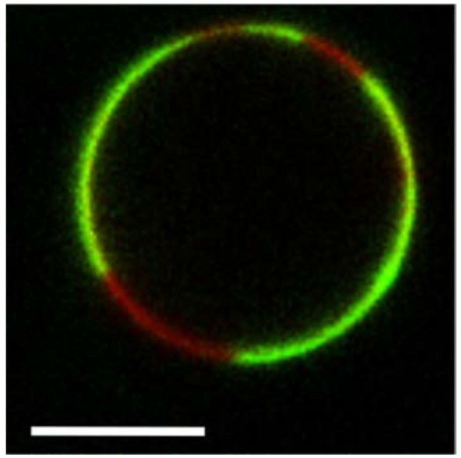
- Membrane-less organelles that behave like liquid droplets
- Enriched in intrinsically disordered proteins (IDPs)
- Example for IDP: RNA-binding protein FUS
- Interaction of FUS-droplets with GUVs, two subsequent wetting transitions:



dewetting for high salt



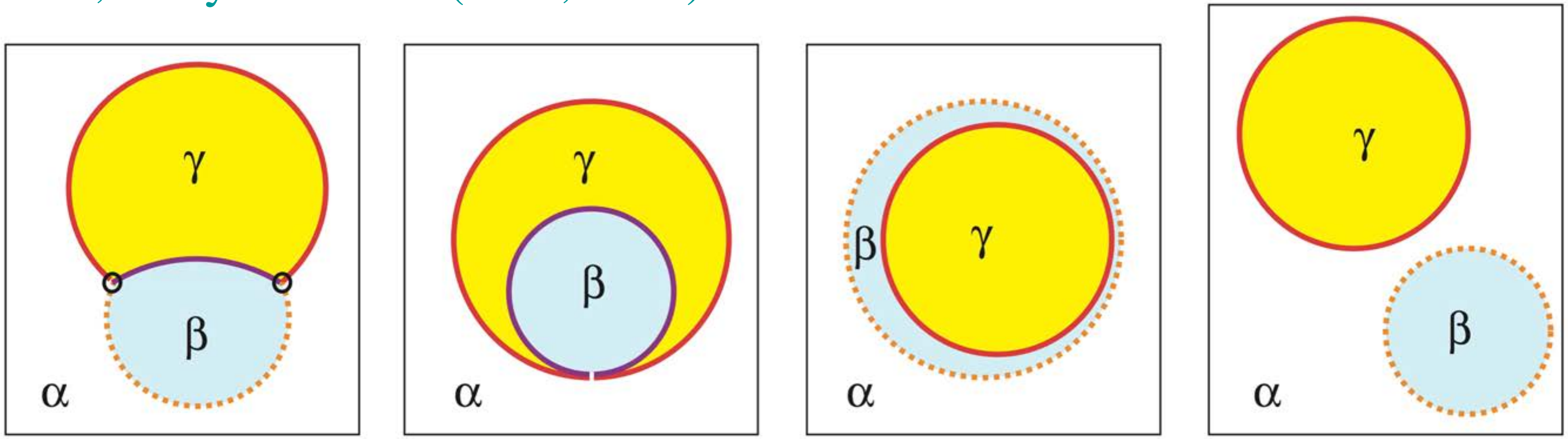
partial wetting for intermediate salt



complete wetting for low salt

Shaping by Capillary Forces

RL, J Phys Chem B (Febr, 2018)

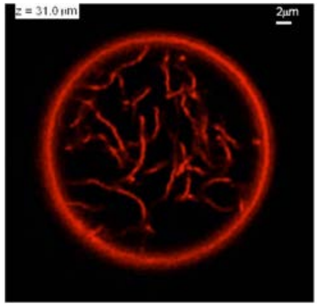


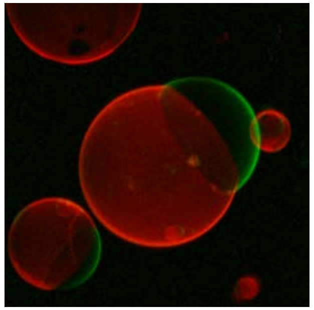
- Apparent and intrinsic contact angles
- Spherical membrane segments imply relation

$$M_{\gamma\alpha}\left(\frac{\Sigma^{\text{eff}}_{\gamma\alpha}}{\Sigma_{\alpha\beta}} - \frac{\sin\theta^{\text{ap}}_{\beta}}{\sin\theta^{\text{ap}}_{\gamma}}\right) = M_{\gamma\beta}\left(\frac{\Sigma^{\text{eff}}_{\gamma\beta}}{\Sigma_{\alpha\beta}} - \frac{\sin\theta^{\text{ap}}_{\alpha}}{\sin\theta^{\text{ap}}_{\gamma}}\right)$$

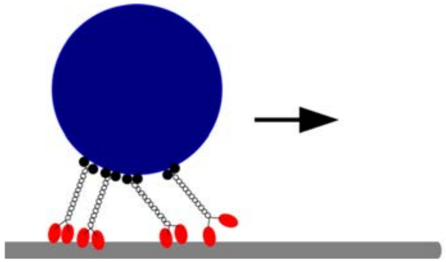
- Effective membrane tensions involve spont curv

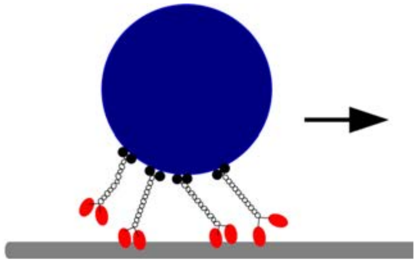
Basic Modules for Synthetic Cells



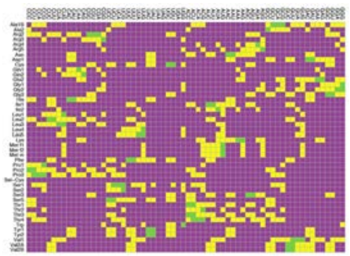


- Membrane and vesicles, fluid compartments, remodeling



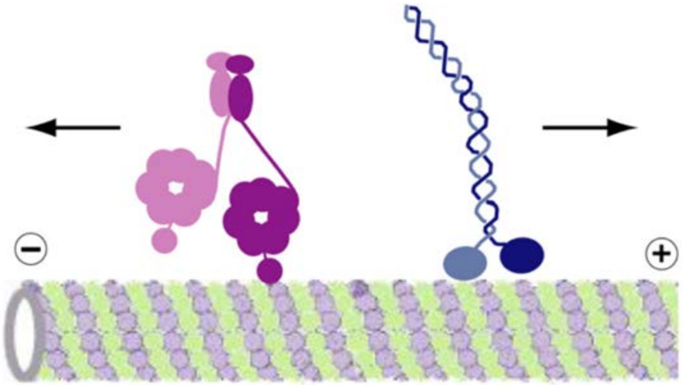


- Directed transport by molecular motors, free energy transduction

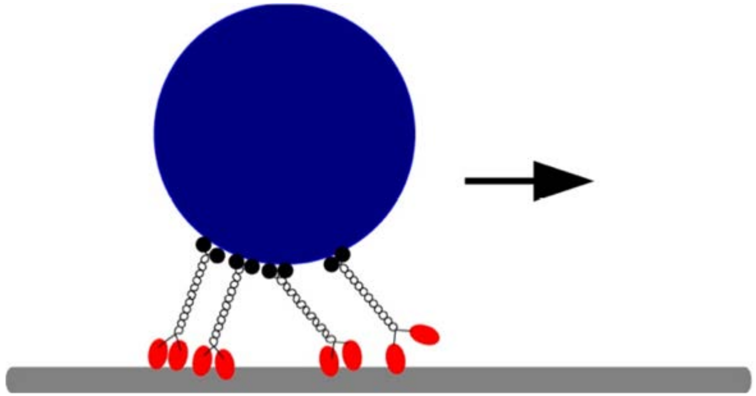


- Template-directed assembly, ribosomes, protein synthesis

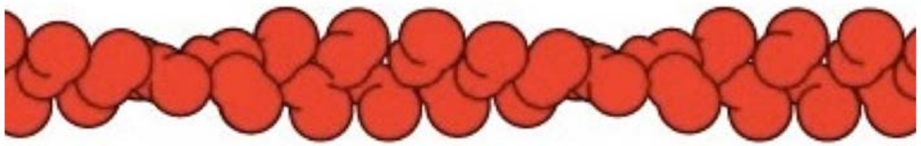
Biomolecular Machines



• Intro: Stepping motors



• Transport: Motor teams

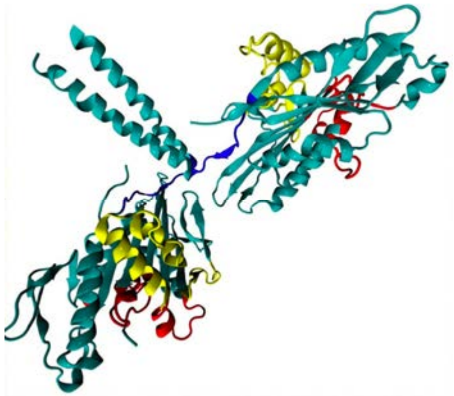


• Structural remodelling: Actin filaments

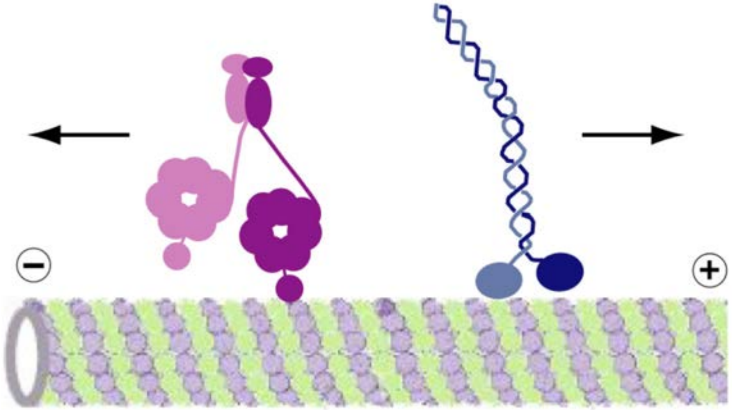
• Information processing: Ribosomes

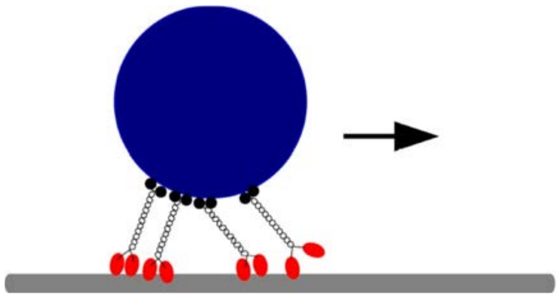
Multiscale Aspects of Mol Motors

• ATP hydrolysis ~ 1 nm



• Mechanical steps ~ 10 nm





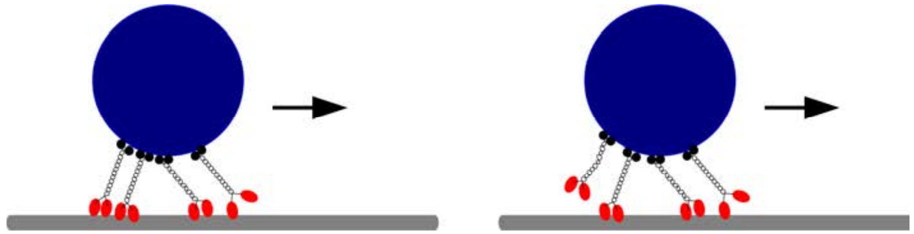
• Cargo transport by motor teams ~ 100 μm

•Traffic of many motors/cargos and phase transitions

Cargo Transport by Motor Teams

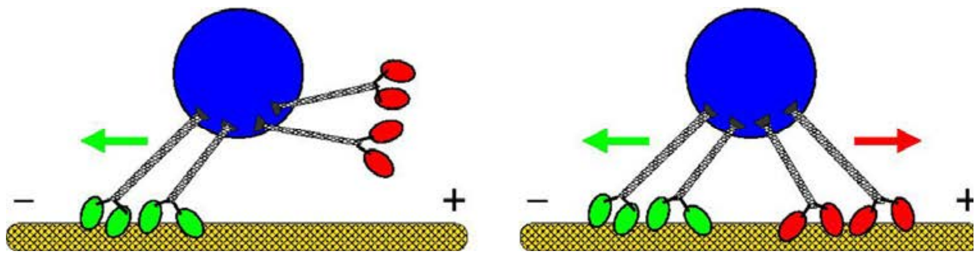
- Transport by N identical motors

  Klumpp and RL, *PNAS* (2005)



- Transport by two antagonistic motor teams, Stochastic tug-of-war

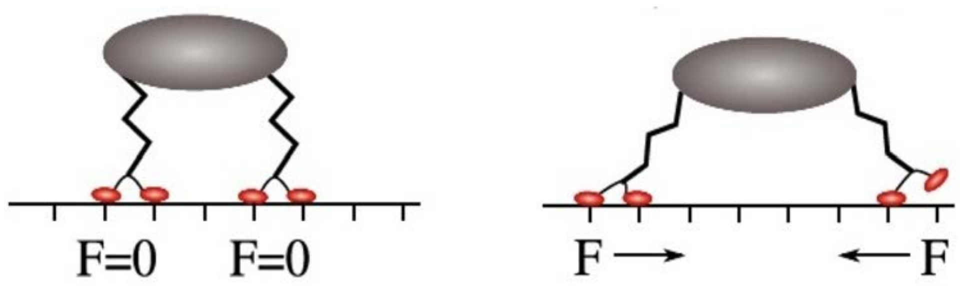
  M. Müller et al, *PNAS* (2008)



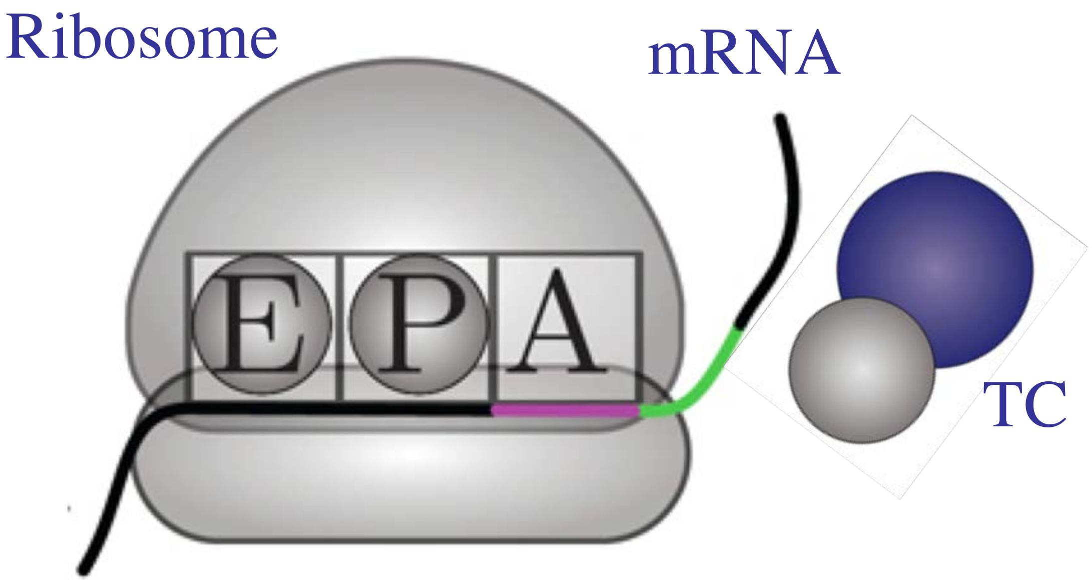
- Elastic linkers between motors and cargo

  Berger et al, *PRL* (2012)

  Ucar, RL, *Soft Matter* (2017)



Protein Synthesis by Ribosomes



TC = ternary complex = tRNA + EF-Tu + GTP

EF-Tu = most abundant protein

- Ribosome steps along codons of mRNA (purple -> green) consuming one ternary complex at each codon
- Elongation cycle during one step:

  Decoding of codon by binding/accommodation of tRNA

  Elongation of growing peptide chain by one amino acid

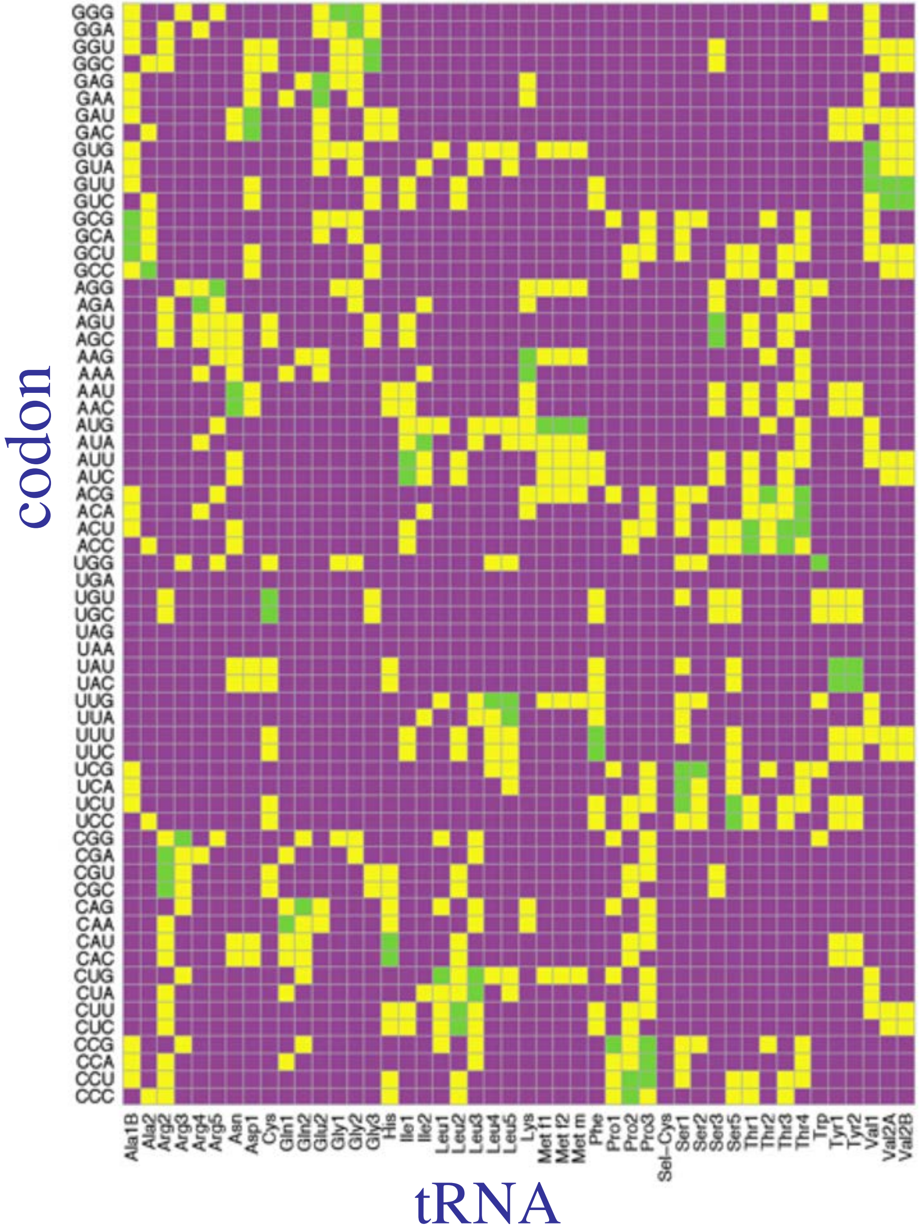
  Translocation of mRNA together with two tRNAs

Codon-tRNA Relationships

- red/purple = non-cognate
  released after initial binding
- yellow = near-cognate
  decoding => wrong amino acid
- green = cognate
  decoding => correct amino acid

- ‚Ocean‘ of non-cognates
  with some near-cognates
  and a few cognates

Decoding pattern

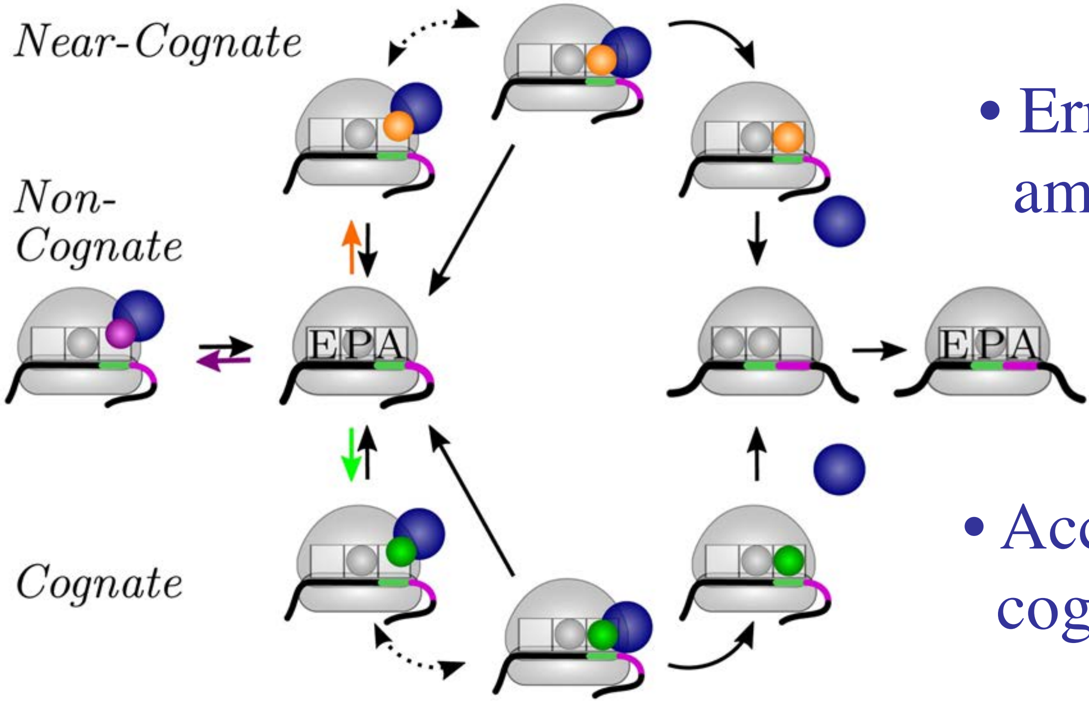


Single Elongation Cycle

Rudorf, Thommen, Rodnina, RL,
PLoS Comp Biol (2014)

- Three branches for tRNA binding:

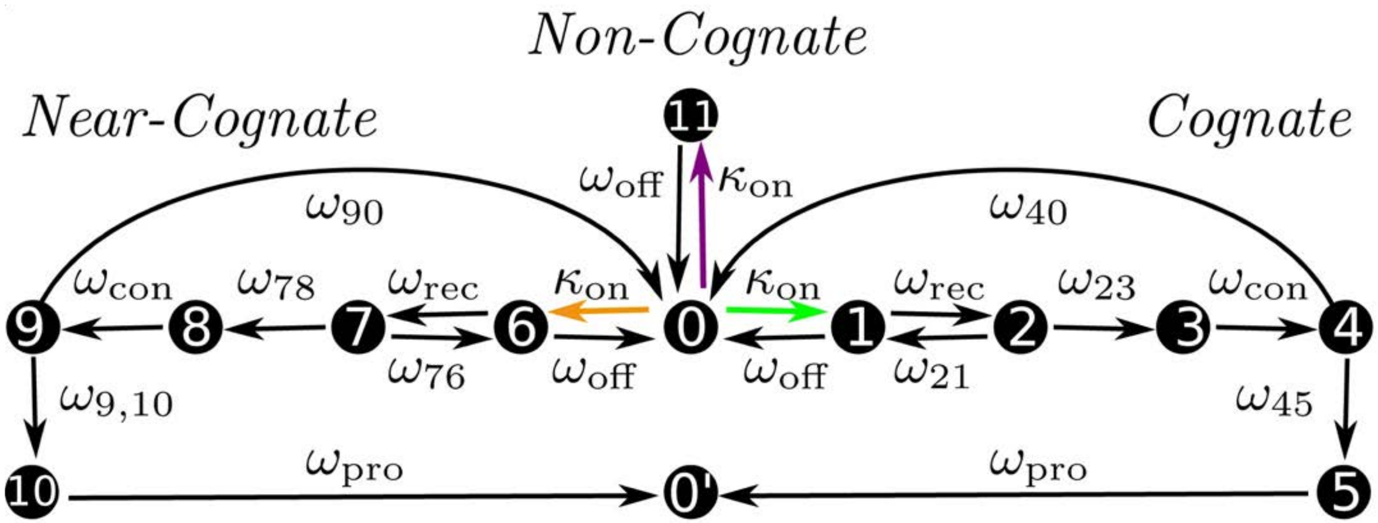
Sophia Rudorf



- Error: wrong amino acid
- Accommodation of cognate tRNA
- Competition between cognate, near-cognate, and non-cognate tRNAs

Markov Process

- Map cartoon of multistep process onto Markov chain:



- Individual transitions:

  initial binding, recognition, initial selection, GTP hydrolysis, phosphate release, proof reading, full accommodation

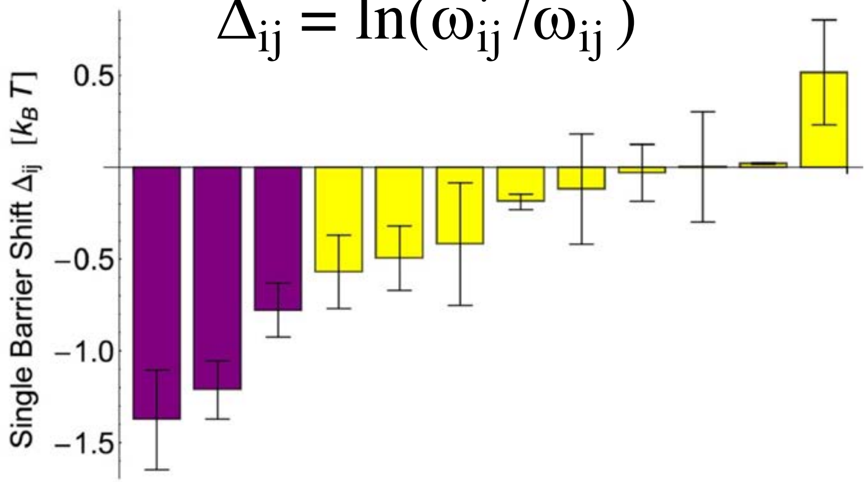
- All transition rates ω_{ij} have been measured in vitro
- Some rates identical for both cognates and near-cognates

From In-Vitro to In-Vivo Rates

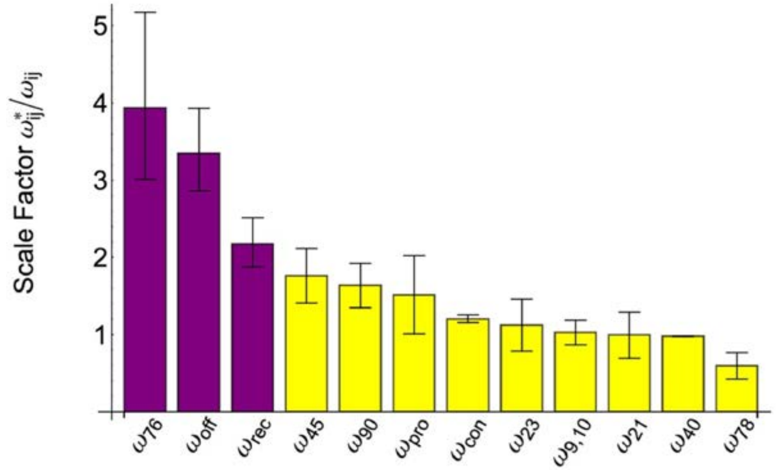
Rudorf, Thommen, Rodnina, RL, *PLoS Comp Biol* (2014)

- Single barrier shifts

$$\Delta_{ij} = \ln(\omega^*_{ij}/\omega_{ij})$$



- Scale factors $\omega^*_{ij}/\omega_{ij}$



- Three in-vivo rates (purple) are significantly increased:

rejection rate ω_{76} for near cognates

dissociation rate ω_{off} after initial binding

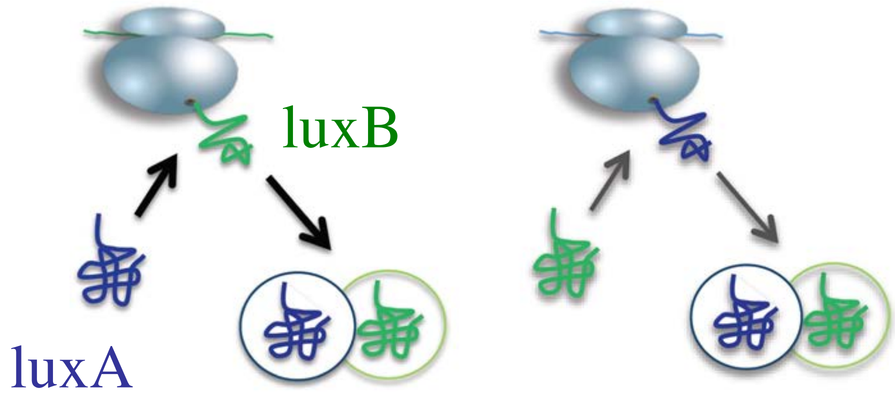
recognition rate ω_{rec} for cognates and near-cognates

Assembly of Protein Complexes

Shieh ... Kramer, Bukau, *Science* (2015)

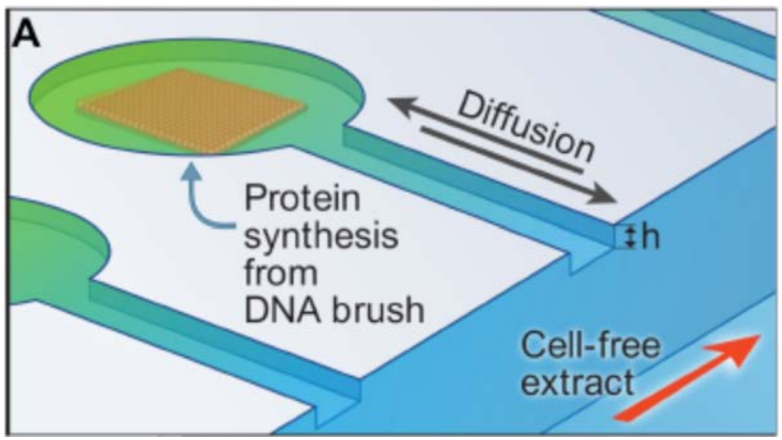
- Co-translational assembly:

  in vivo synthesis of two proteins,
  assembly during translation,
  luxA binds to emerging luxB



- Protein synthesis on a chip:

  different DNA compartments
  for different proteins,
  control of spatial separation
  between different compartments

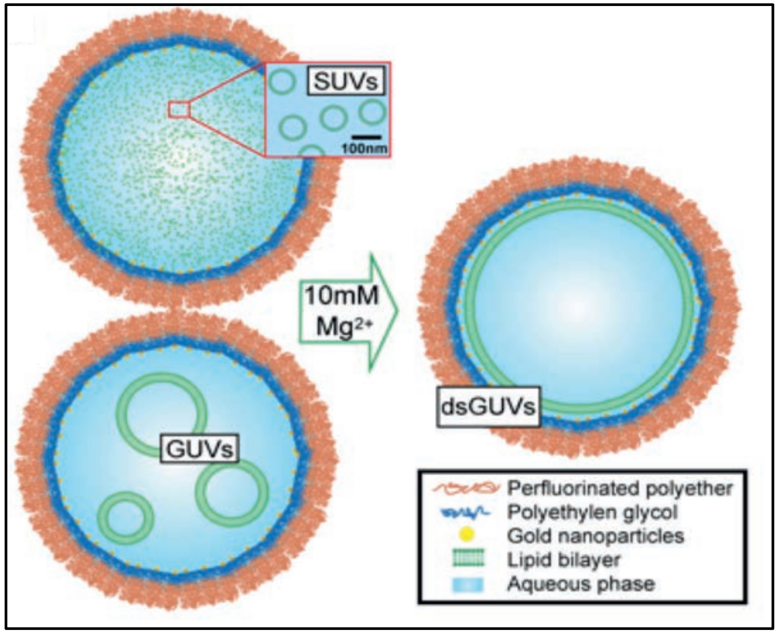


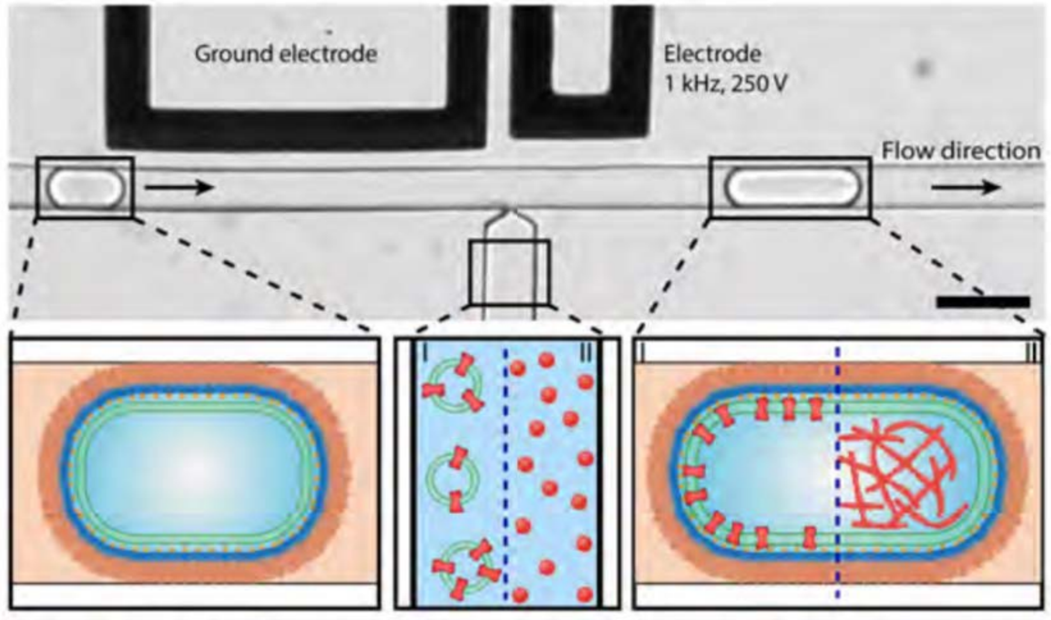
Karzbrun ... Bar-Ziv, *Science* (2014)

Sequential Bottom-Up Assembly

Weiss et al, *Nature Materials* (Nov. 2017)

- Four MPIs within MaxSynBio, leading PI: Joachim Spatz





- Water-in-Oil emulsion droplets
- Formation of GUV supported by the droplet surface

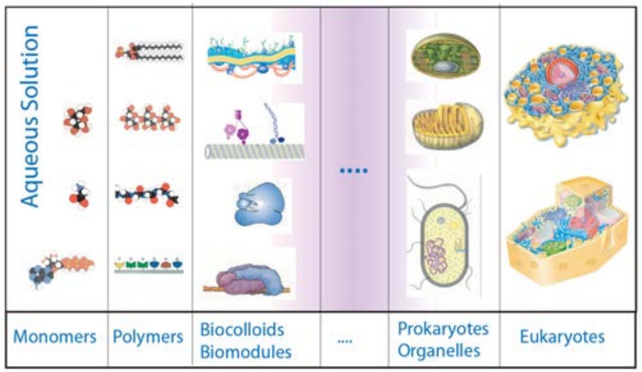
- Additional components by pico-injection
- Example: ATP synthase

Perspectives and Challenges

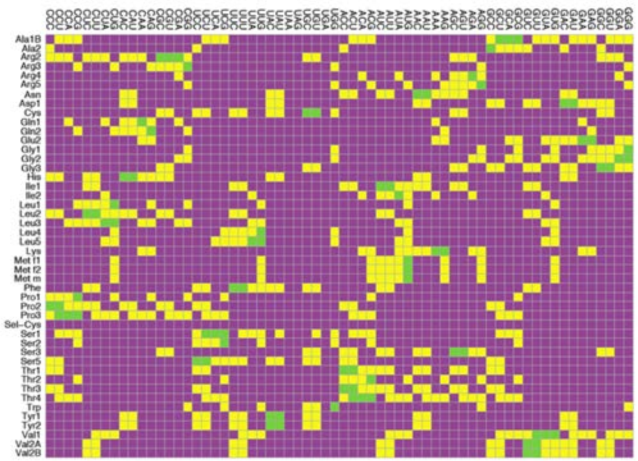
- Further steps of sequential assembly:

  Compartments + ATP synthase + filaments + motors + ...

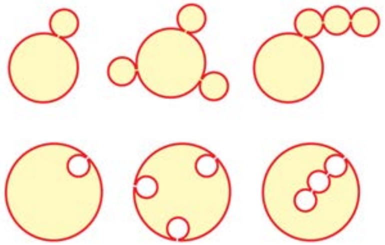
- Importance of ionic conditions
- Unwanted interactions, complexes
- Alternative assembly pathways



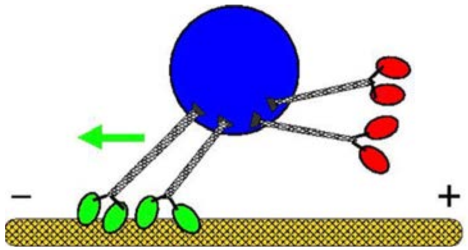
- Evolution via selection (failures)
- Evolution as a learning process
- Ancestor cells after ~ 10^8 years
- Synthetic cells after ??? years
- Persistent complexity gap ?



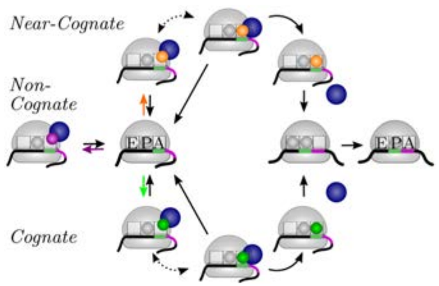
Summary



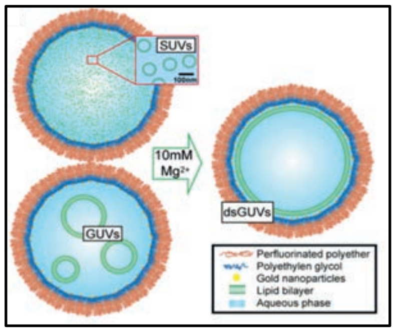
- Membrane compartments, multiresponsive, many architectures



- Molecular motors, cargo transport and concentr gradients



- Protein synthesis, comparison of in vivo and in vitro



- Droplet-stabilized GUVs, new platform for sequential assembly

Coworkers

- Membranes

  Rumiana Dimova
  Tom Robinson
  Jaime Agudo-Canalejo
  Tripta Bhatia
  Yunuen Avalos Padillo
  Jan Steinkühler

- Motors + Ribosomes

  Stefan Klumpp
  Sophia Rudorf
  Mehmet Ucar
  Stefanie Foerste
  Nadin Haase
  Simon Christ

- Collaborations

  Marina Rodnina
  Joachim Spatz
  Tony Hyman
  Titus Franzmann
  Günther Kramer
  Roy Bar-Ziv